REMARKS/ARGUMENTS

The foregoing amendments in the specification and claims are of a formal nature, and do not add new matter.

Prior to the present amendment, Claims 33, 38-40 and 44-54 were pending in this application and were rejected on various grounds. With this amendment, Claims 48-54 have been canceled.

Claims 33, 38-40 and 44-47 are pending after entry of the instant amendment. Applicants expressly reserve the right to pursue any canceled matter in subsequent continuation, divisional or continuation-in-part applications.

Priority

The Examiner states that "Applicant has identified support for the claim in U.S. Provisional Application Serial No. 60/162,506, filed October 29, 1999, in which the utility is first asserted." The Examiner further asserts that "no priority would be given to U.S. Provisional Application Serial No. 60/100,661, where no utility for the nucleic acid is provided".

Applicants respectfully disagree.

Applicants rely on the gene amplification assay (Example 143) for patentable utility which was first disclosed in U.S. Provisional Application Serial No. 60/162,506, filed October 29, 1999, priority to which has been claimed in this application. All claim limitations are fully supported by the disclosure of U.S. Provisional Application Serial No. 60/162,506, filed on October 29, 1999, which is specific for the PRO1269 polypeptide and its coding sequence. In support, Applicants enclosed herewith pages 113-149 of U.S. Provisional Application Serial No. 60/162,506, filed October 29, 1999, which shows the gene amplification assay.

Further, the PRO1269 polypeptide sequence and its encoding nucleic acid sequence were first disclosed in the U.S. Provisional Application Serial No. 60/100,661, filed on September 16, 1998, priority to which has been claimed in this application.

Claim Rejections – 35 U.S.C. §112, First Paragraph

Claims 48-54 were rejected under 35 U.S.C. §112, first paragraph, because, according to

the rejection, the specification, "while being enabling for the specific PRO1269 sequence of SEQ ID NO:215, does not reasonably provide enablement for anything which encodes SEQ ID NO:216, which hybridizes to SEQ ID NO:215 or which shares some percent identity."

Applicants respectfully submit that the cancellation of Claims 48-54 renders the rejection of these claims moot. Hence, the present rejection should be withdrawn.

Claim Rejections – 35 U.S.C. §112, First Paragraph (Written Description)

Claims 48-54 have been rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicants respectfully submit that the cancellation of Claims 48-54 renders the rejection of these claims moot. Hence, the present rejection should be withdrawn.

Claim Rejections - 35 U.S.C. §102

Claims 33, 38-40 and 44-54 are rejected under 35 U.S.C. §102(e) as allegedly being anticipated by Young *et al.*, (U.S. Patent No. 6,444,790, effective priority date December 23, 1998).

Applicants respectfully submit that the cancellation of Claims 48-54 renders the rejection of these claims moot.

Applicants have claimed priority to U.S. Provisional Application Serial No. 60/100,661, filed on September 16, 1998. The present application is entitled to the priority date of September 16, 1998, which precedes, at least by three months, the earliest priority date of Young *et al.* (December 23, 1998).

Further, Applicants respectfully submitted signed copies of the Declarations under 37 C.F.R. §1.131 by Dr. Botstein, Dr. Goddard, Dr. Godowski, Dr. Gurney, Ms. Roy, Mr. Watanabe and Dr. Wood on November 23, 2004 that establishes that Applicants had cloned, sequenced and homology to granulocyte peptide A identified before the prior art date of December 23, 1998.

U.S. Provisional Application Serial No. 60/100,661 simply needs to disclose what is disclosed in the cited reference to support the priority claim

Applicants respectfully submit that in order to overcome the 35 U.S.C. §102(e) rejection over Young *et al.*, the Declaration by Dr. Botstein, Dr. Goddard, Dr. Godowski, Dr. Gurney, Ms. Roy, Mr. Watanabe and Dr. Wood ("Declaration") simply needs to provide a disclosure commensurate in scope with the disclosure in the prior art document by Young *et al.* to support the priority claim.

The Examiner contends that Stempel "states in relevant part 'unless the reference also teaches how to use the compound it describes (see page [12] of the response).' This is precisely that situation. It is undisputed, and actually admitted by Applicant, that their U.S. Provisional Application Serial No. 60/100,661 does not provide any utility for the claimed sequence." Furthermore, the Examiner asserts that "Young is a reference that also teaches how to use the compound it describes.... The Young patent is literally identical to the provisional from which it depends (U.S. Provisional Application Serial No. 60/113,809). The Young patent provides identical utilities for the claimed SEQ ID NO:4 and for the sequence at issue, SEQ ID NO:6. Since issued patents are PRESUMED useful and enabled, and no evidence overcoming that presumption has been presented, Young is presumptively enabled for SEQ ID NO:6 simply based on the fact that the patent issued." (See page 13 of the instant Office Action).

Applicants respectfully disagree and traverse the rejection.

The Examiner's conclusions concerning the presumption of utility and enablement for SEQ ID NO:6 in U.S. Patent No. 6,444,790 (Young *et al.*) "simply based on the fact that the patent issued" are flawed for several reasons. First of all, as the Examiner has admitted above, the issued claims of the Young *et al.* patent are directed to isolated proteins comprising various amino acid residues of SEQ ID NO:4 and not SEQ ID NO:6. Since the presumption of validity applies only to the subject matter covered by the claims of an issued patent, contrary to the Examiner's assertion, the utility for SEQ ID NO:6 cannot be presumed based on the fact that the Young *et al.* patent issued with claims covering proteins other than SEQ ID NO:6. Secondly, even if the patent had issued with claims covering SEQ ID NO:6 (as it had not), the presumption

of validity would be rebutted by the fact that the disclosure of Young *et al.* is completely devoid of any teaching of a real life utility for this molecule.

The Examiner alleges that "Young teaches specific diagnosis of specific disorders including wound healing at column 6, lines 48-67. This is a specific and substantial utility, unlike those presented in the current application.... The specification expressly states this diagnostic ability and the differential expression of the protein during wound healing. Diagnosing problems in wound healing is clearly a credible, specific and substantial utility." Applicants respectfully point out to the Examiner that the Young patent teaches three peptidoglycan recognition protein-related proteins expressed by keratinocytes, would-healing tissues and chondrosarcoma tissue, referred to as PGRP-K (Keratinocytes), PGRP-W (Wound-healing) and PGRP-C (Chondrosarcoma), respectively. (See Abstract). The amino acid sequence of PGRP-K is shown in SEQ ID NO:2, the amino acid sequence of PGRP-W is shown in SEQ ID NO:4 and the amino acid sequence of PGRP-C is shown in SEQ ID NO:6. Therefore, a careful reading of the Young patent shows that comments relating to various disorders wherein a higher or lower levels of the gene expression may be detected in the wound healing tissues is specifically directed to PGRP-W, SEQ ID NO:4 and not PGRP-C, SEQ ID NO:6. More specifically, the Young patent teaches that:

> the nucleic acid molecule described in FIG. 1 (SEQ ID NO:1) was discovered in a cDNA library derived from Human keratinocytes, the nucleic acid molecule described in FIG. 2 (SEQ ID NO:3) was discovered in cDNA libraries derived from Human keratinocytes and Human tissues undergoing wound-healing, and the nucleic acid molecule described in FIG. 3 (SEQ ID NO:5) was discovered in a cDNA libraries derived from Human chondrosarcoma.

In addition, Applicants note that the Young *et al.* patent teaches, "PGRP-W is 42% homologous to PGRP-C, and PGRP-K is 39% homologous to PGRP-C." (See column 10, lines 8-9 of U.S. Patent No. 6,444,790). Therefore, it would appear that PGRP-C has rather low sequence identity to both PGRP-W and PGRP-K. Thus, based solely on homology, a person skilled in the art at the priority date of that application would not have reasonably concluded that PGRP-W and PGRP-C would have the same utility.

Hence, the disclosure for "diagnosing problems in wound healing" in the Young patent does not support a diagnostic utility for the sequence at issue, SEQ ID NO:6.

Applicants further maintain the position that the Young patent is devoid of any experimental data demonstrating the biological activity of PGRP-C, or identifying any specific diseases associated with the expression level of this protein or its encoding gene. As mentioned above, while Young discloses a protein designated PGRP-C and provides sequence homology to both human peptidoglycan recognition protein (PGRP) as well as murine Tag-7, it does not provide any specific experimental data to support the utility in diagnosing various disorders by assaying the PGRP-C gene expression levels. All of the teachings in the Young patent regarding such utility is merely speculative and entirely dependent on the fact that the nucleic acid encoding PGRP-C polypeptide was discovered in cDNA libraries derived from Human chondrosarcoma. (See column 9, lines 45-48). Column 60, line 57 states, "Cells which express either the PGRP-K, PGRP-W and/or PGRP-C polypeptides are believed to have a potent cellular response to infection ...." Similarly, Column 61, line 16 states,

> Thus it is believed that certain tissues in mammals with certain diseases and infections...., diseases associated with increased or decreased cell survival, ... express significantly altered (*e.g., enhanced or decreased*) levels of either the PGRP-K, PGRP-W and/or PGRP-C polypeptides when compared to a corresponding "standard" mammal.

(Emphasis added). Accordingly, the Young patent is devoid any experimental support that would show how PGRP-C can be used to diagnose any disorders or diseases. It merely suggests that PGRP-C may be useful in diagnosing certain disorders/diseases.

Applicants respectfully submit that U.S. Provisional Application Serial No. 60/100,661, filed on September 16, 1998, similarly provides the nucleic acid and amino acid sequences of the PRO1269 polypeptide and the homology of the polypeptide to the bovine granulocyte peptide A (see U.S. Provisional Application Serial No. 60/100,661 on page 15, under the section titled "Full-length PRO1269"). Furthermore, considering its homology to the granulocyte peptide A, Applicants also suggest the PRO1269 polypeptide to be newly identified member of the granulocyte A peptide family and may possess biological activity typical of that family of

peptides.

Applicants respectfully submit that the Young patent only discloses a polynucleotide sequence (SEQ ID NO:6), its encoding nucleic acid sequence (SEQ ID NO:5) and a sequence homology. Therefore, the Declaration simply needs to show possession of the polypeptide sequence, its encoding polynucleotide sequence as disclosed in the Young *et al.* patent, and a sequence homology in order to overcome the 35 U.S.C. §102 rejection.

The Declaration clearly states that U.S. Provisional Application Serial No. 60/100,661, filed on September 16, 1998, discloses sequences designated as SEQ ID NO:2 and SEQ ID NO:1, which are identical to SEQ ID NO:215 and SEQ ID NO:216, respectively, of the above-identified application. Further, the Declaration confirms that U.S. Provisional Application Serial No. 60/100,661, filed on September 16, 1998, discloses that SEQ ID NO:1, corresponding to SEQ ID NO:216 of the above-identified application, has homology to granulocyte peptide A.

Accordingly, Applicants respectfully submit that the disclosures are commensurate in scope and that U.S. Provisional Application Serial No. 60/100,661, filed on September 16, 1998, discloses all that the cited prior art discloses.

Consequently, based on the holdings of *In re* Stempel and *In re* Moore, Applicants respectfully submit that Young *et al.* is not prior art under 102(e) since its effective priority date is after the invention by the Applicants for patent. Accordingly, the Examiner is respectfully requested to reconsider and withdraw the rejection of Claims 33, 38-40 and 44-47 under 35 U.S.C. §102(e).

Claims 33 and 48-54 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Kang *et al.*, (Proc. Natl. Acad. Sci. (August 1998) 92:10078-10082)) and Genbank Accession No. AF076483 (August 15, 1998). In particular, the Examiner asserts that "Kang's sequence comprises the full length coding sequence 'from within' SEQ ID NO:215".

Applicants respectfully disagree and traverse the rejection.

Applicants respectfully submit that the cancellation of Claims 48-54 renders the rejection of these claims moot.

Applicants respectfully point out to the Examiner that the Kang's sequence (AF076483) does not have 100% sequence identity within the entire coding sequence of SEQ ID NO:215. (See page 11 of the instant Office Action, e.g., mismatch at position 61 of SEQ ID NO:215 (Query sequence)). Therefore, Applicants respectfully submit that Claim 33 is not anticipated by Kang *et al.* Accordingly, the Examiner is respectfully requested to reconsider and withdraw the rejection of Claim 33 under 35 U.S.C. §102(b).

CONCLUSION

In conclusion, the present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited. Should there be any further issues outstanding, the Examiner is invited to contact the undersigned attorney at the telephone number shown below. Please charge any additional fees, including fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641** (referencing Attorney's Docket No. **39780-2830 P1C53**). Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: January 7, 2005

By: _____
Anna L. Barry (Reg. No. 51,436)

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databases which included public EST databases (*e.g.*, GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altshul *et al.*, Methods in Enzymology, 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a consensus DNA sequence with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence is herein designated DNA56374.

In light of the sequence homology between the DNA56374 sequence and Incyte EST no. 2855769, Incyte EST no. 2855769 was purchased and the cDNA insert was obtained and sequenced. Incyte EST no. 2855769 was derived from a library constructed from female breast fat tissue. The sequence of this cDNA insert is herein designated as DNA73744-1665.

The entire coding sequence is included in Figure 33 (SEQ ID NO:48). Clone DNA73744-1665 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 90-92 and ending at the stop codon at nucleotide positions 828-830 (Figure 33). The predicted polypeptide precursor is 246 amino acids long (Figure 34; SEQ ID NO:49). The full-length PRO1555 protein shown in Figure 34 has an estimated molecular weight of about 26,261 daltons and a pI of about 5.65. Analysis of the full-length PRO1555 sequence shown in Figure 34 (SEQ ID NO:49) evidences the presence of a variety of important polypeptide domains, wherein the locations given for those important polypeptide domains are approximate as described above. Analysis of the full-length PRO1555 sequence shown in Figure 34 evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 31; transmembrane domains from about amino acid 11 to about amino acid 32 and from about amino acid 195 to about amino acid 217; a potential N-glycosylation site from about amino acid 111 to about amino acid 115; potential casein kinase II phosphorylation sites from about amino acid 2 to about amino acid 6, from about amino acid 98 to about amino acid 102, and from about amino acid 191 to about amino acid 195; and potential N-myristoylation sites from about amino acid 146 to about amino acid 152, and from about amino acid 192 to about amino acid 198. Clone DNA73744-1665 has been deposited with ATCC on October 6, 1998 and is assigned ATCC deposit no. 203322.

An analysis of the Dayhoff database (version 35.45 SwissProt 35), using a WU-BLAST2 sequence alignment analysis of the full-length sequence shown in Figure 34 (SEQ ID NO:49), evidenced some homology between the PRO1555 amino acid sequence and the following Dayhoff sequences: YKA4_CAEEL, AB014541_1, HVSX99518_2, SSU63019_1, GEN14286, MMU68267_1, XP2_XENLA, ICP4_HSV11, P_W40200, and AE001360_1.

EXAMPLE 20

Gene Amplification

This example shows that the PRO381-, PRO1269-, PRO1410-, PRO1755-, PRO1780-, PRO1788-, PRO3434-, PRO1927-, PRO3567-, PRO1295-, PRO1293-, PRO1303-, PRO4344-, PRO4354-, PRO4397-, PRO4407-, PRO1555-, PRO1096-, PRO2038- or PRO2262-encoding genes are amplified in the genome of certain human lung, colon and/or breast cancers and/or cell lines. Amplification is associated with overexpression of the

gene product, indicating that the polypeptides are useful targets for therapeutic intervention in certain cancers such as colon, lung, breast and other cancers. Therapeutic agents may take the form of antagonists of PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344, PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262 polypeptides, for example, murine-human chimeric, humanized or human antibodies against a PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344, PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262 polypeptide.

The starting material for the screen was genomic DNA isolated from a variety of cancers. The DNA is quantitated precisely, *e.g.*, fluorometrically. As a negative control, DNA was isolated from the cells of ten normal healthy individuals which was pooled and used as assay controls for the gene copy in healthy individuals (not shown). The 5' nuclease assay (for example, TaqMan™) and real-time quantitative PCR (for example, ABI Prizm 7700 Sequence Detection System™ (Perkin Elmer, Applied Biosystems Division, Foster City, CA)), were used to find genes potentially amplified in certain cancers. The results were used to determine whether the DNA encoding PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344, PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262 is over-represented in any of the primary lung or colon cancers or cancer cell lines or breast cancer cell lines that were screened. The primary lung cancers were obtained from individuals with tumors of the type and stage as indicated in Table 4. An explanation of the abbreviations used for the designation of the primary tumors listed in Table 4 and the primary tumors and cell lines referred to throughout this example has been given hereinbefore.

The results of the TaqMan™ are reported in delta (Δ) Ct units. One unit corresponds to 1 PCR cycle or approximately a 2-fold amplification relative to normal, two units corresponds to 4-fold, 3 units to 8-fold amplification and so on. Quantitation was obtained using primers and a TaqMan™ fluorescent probe derived from the PRO381-, PRO1269-, PRO1410-, PRO1755-, PRO1780-, PRO1788-, PRO3434-, PRO1927-, PRO3567-, PRO1295-, PRO1293-, PRO1303-, PRO4344-, PRO4354-, PRO4397-, PRO4407-, PRO1555-, PRO1096-, PRO2038- or PRO2262-encoding gene. Regions of PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344, PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262 which are most likely to contain unique nucleic acid sequences and which are least likely to have spliced out introns are preferred for the primer and probe derivation, *e.g.*, 3'-untranslated regions. The sequences for the primers and probes (forward, reverse and probe) used for the PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344, PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262 gene amplification analysis were as follows:

PRO381 (DNA44194-1317)

44194.tm.f:

5'-CTTTGAATAGAAGACTTCTGGACAATTT-3' (SEQ ID NO:56)

44194.tm.p:

5'-TTGCAACTGGGAATATACCACGACATGAGA-3' (SEQ ID NO:57)

44194.tm.r:

5'-TAGGGTGCTAATTTGTGCTATAACCT-3' (SEQ ID NO:58)

44194.tm.f2:

5'-GGCTCTGAGTCTCTGCTTGA-3' (SEQ ID NO:59)

44194.tm.p2:

5'-TCCAACAACCATTTTCCTCTGGTCC-3' (SEQ ID NO:60)

44194.tm.r2:

5'-AAGCAGTAGCCATTAACAAGTCA-3' (SEQ ID NO:61)

PRO1269 (DNA66520-1536)

66520.tm.f1:

5'-AAAGGACACCGGGATGTG-3' (SEQ ID NO:62)

66520.tm.p1:

5'-AGCGTACACTCTCTCCAGGCAACCAG-3' (SEQ ID NO:63)

66520.tm.r1:

5'-CAATTCTGGATGAGGTGGTAGA-3' (SEQ ID NO:64)

PRO1410 (DNA68874-1622)

68874.tm.f1:

5'-CAGGACTGAGCGCTTGTTTA-3' (SEQ ID NO:65)

68874.tm.p1:

5'-CAAAGCGCCAAGTACCGGACC-3' (SEQ ID NO:66)

68874.tm.r1:

5'-CCAGACCTCAGCCAGGAA-3' (SEQ ID NO:67)

PRO1755 (DNA76396-1698)

76396.tm.f1:

5'-TCATGGTCTCGTCCCATTC-3' (SEQ ID NO:68)

76396.tm.p1:

5'-CACCATTTGTTTCTCTGTCTCCCCATC-3' (SEQ ID NO:69)

76396.tm.r1:

5'-CCGGCATCCTTGGAGTAG-3' (SEQ ID NO:70)

PRO1780 (DNA71169-1709)

71169.tm.f1:

5'-CTCTGGTGCCCACAGTGA-3' (SEQ ID NO:71)

71169.tm.p1:

5'-CCATGCCTGCTCAGCCAAGAA-3' (SEQ ID NO:72)

71169.tm.r1:

5'-CAGGAAATCTGGAAACCTACAGT-3' (SEQ ID NO:73)

PRO1788 (DNA77652-2505)

77652.tm.f1:

5'-TCCCCATTAGCACAGGAGTA-3' (SEQ ID NO:74)

77652.tm.p1:

5'-AGGCTCTTGCCTGTCCTGCTGCT-3' (SEQ ID NO:75)

77652.tm.r1:

5'-GCCCAGAGTCCCACTTGT-3' (SEQ ID NO:76)

PRO3434 (DNA77631-2537)

77631.tm.f1:

5'-GTCCAGCAAGCCCTCATT-3' (SEQ ID NO:77)

77631.tm.p1:

5'-CTTCTGGGCCACAGCCCTGC-3' (SEQ ID NO:78)

77631.tm.r1:

5'-CAGTTCAGGTCGTTTCATTCA-3' (SEQ ID NO:79)

PRO1927 (DNA82307-2531)

82307.tm.f1:

5'-CCAGTCAGGCCGTTTTAGA-3' (SEQ ID NO:80)

82307.tm.p1:

5'-CGGGCGCCCAAGTAAAAGCTC-3' (SEQ ID NO:81)

82307.tm.r1:

5'-CATAAAGTAGTATATGCATTCCAGTGTT-3' (SEQ ID NO:82)

PRO3567 (DNA56049-2543)

56049.tm.f1:

5'-GGAAATGGTCTCAAGGGAAA-3' (SEQ ID NO:83)

56049.tm.p1:

5'-TCACTTTGACCCTGTCTTGGAACGTC-3' (SEQ ID NO:84)

56049.tm.r1:

5'-GGTAGAATTCCAGCATTTGGTA-3' (SEQ ID NO:85)

PRO1295 (DNA59218-1559)

59218.tm.f1:

5'-AGGACTTGCCCTCAGGAA-3' (SEQ ID NO:86)

59218.tm.r1:

5'-CGCAGGACAGTTGTGAAAATA-3' (SEQ ID NO:87)

59218.tm.p1:

5'-ATGACGCTCGTCCAAGGCCAC-3' (SEQ ID NO:88)

PRO1293 (DNA60618-1557)

60618.tm.f1:

5'-CCCACCTGTACCACCATGT-3' (SEQ ID NO:89)

60618.tm.p1:

5'-ACTCCAGGCACCATCTGTTCTCCC-3' (SEQ ID NO:90)

60618.tm.r1:

5'-AAGGGCTGGCATTCAAGTU-3' (SEQ ID NO:91)

PRO1303 (DNA65409-1566)

65409.tm.f1:

5'-CTGGCCCTCAGAGCACCAAT-3' (SEQ ID NO:92)

65409.tm.p1:

5'-TCCTCCATCACTTCCCCTAGCTCCA-3' (SEQ ID NO:93)

65409.tm.r1:

5'-CTGGCAGGAGTTAAAGTTCCAAGA-3' (SEQ ID NO:94)

PRO4344 (DNA84927-2585)

84927.tm.f1:

5'-GGGCAACAGCCTGAGAGT-3' (SEQ ID NO:95)

84927.tm.p1:

5'-ACTCAGTGTTGATTCTCTATCGTGATGCG-3' (SEQ ID NO:96)

84927.tm.r1:

5'-GAGCAGCAGGCATCAATTT-3' (SEQ ID NO:97)

PRO4354 (DNA92256-2596)

92256.tm.f1:

5'-GGCCTGGAGTTGCTGATAA-3' (SEQ ID NO:98)

92256.tm.p1:

5'-TTGAGCTTAAGTAGACCAAGTATCTATCCCACCTAAA-3' (SEQ ID NO:99)

92256.tm.r1

5'-GGTGGGCTCTGGGTTACA-3' (SEQ ID NO:100)

PRO4397 (DNA83505-2606)

83505.tm.f1:

5'-AGCCGGTTTCCCAGATTAT-3' (SEQ ID NO:101)

83505.tm.p1:

5'-TGCCGTGTATGTGGTTCTTCCCTG-3' (SEQ ID NO:102)

83505.tm.r1:

5'-GAGACAGGCACCTGGTGAT-3' (SEQ ID NO:103)

PRO4407 (DNA92264-2616)

92264.tm.f1:

5'-TGTTTCTGCCTGGACATCA-3' (SEQ ID NO:104)

92264.tm.r1:

5'-GCTTACCGTGGCCTGACT-3' (SEQ ID NO:105)

92264.tm.p1:

5'-TCCTCAGGGTCCAAGTCCCCAT-3' (SEQ ID NO:106)

PRO1555 (DNA73744-1665)

73744.tm.f1:

5'-CCTTGAAAAGGACCCAGTTT-3' (SEQ ID NO:107)

73744.tm.p1:

5'-ATGAGTCGCACCTGCTGTTCCC-3' (SEQ ID NO:108)

73744.tm.r1:

5'-TAGCAGCTGCCCTTGGTA-3' (SEQ ID NO:109)

73744.tm.f2:

5'-AACAGCAGGTGCGACTCATCTA-3' (SEQ ID NO:110)

73744.tm.p2:

5'-TGCTAGGCGACGACACCCAGACC-3' (SEQ ID NO:111)

73744.tm.r2 :

5'-TGGACACGTGGCAGTGGA-3' (SEQ ID NO:112)

PRO1096 (DNA61870)

61870.tm.f1:

5'-TGGACCATGAAGCCAGTTT-3' (SEQ ID NO:113)

61870.tm.p1:

5'-CCTTTTTAGTTGGCTAACTGACCTGGAAAGAA-3' (SEQ ID NO:114)

61870.tm.r1:

5'-TGAATAGTCACTTTGAGGTTATTGC-3' (SEQ ID NO:115)

PRO2038 (DNA83014)

83014.tm.f1:

5'-CCTGGCTCCACCTGTGAT-3' (SEQ ID NO:116)

83014.tm.p1:

5'-ACCTCCCCCTGCTTCCTGCTG-3' (SEQ ID NO:117)

83014.tm.r1:

5'-CCTCAGACCCCATGAGTGA-3' (SEQ ID NO:118)

PRO2262 (DNA88273)

88273.tm.f1:

5'-GAGGAATGGCCCAACAGT-3' (SEQ ID NO:119)

88273.tm.p1:

5'-TGGCAGCCACCCTTCAGTGAG-3' (SEQ ID NO:120)

88273.tm.r1:

5'-CAGCACATCACGTGTCCA-3' (SEQ ID NO:121)

88273.tm.f2:

5'-GAGGAATGGCCCAACAGT-3' (SEQ ID NO:122)

88273.tm.p2:

5'-TGTCCATGCCCCTGGTCCAC-3' (SEQ ID NO:123)

88273.tm.r2 :

5'-GAGGTACAGAGCAGCACATCA-3' (SEQ ID NO:124)

The 5' nuclease assay reaction is a fluorescent PCR-based technique which makes use of the 5' exonuclease activity of Taq DNA polymerase enzyme to monitor amplification in real time. Two oligonucleotide primers are used to generate an amplicon typical of a PCR reaction. A third oligonucleotide, or probe, is designed to detect nucleotide sequence located between the two PCR primers. The probe is non-extendible by Taq DNA polymerase enzyme, and is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe. During the amplification reaction, the Taq DNA polymerase enzyme cleaves the probe in a

template-dependent manner. The resultant probe fragments disassociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore. One molecule of reporter dye is liberated for each new molecule synthesized, and detection of the unquenched reporter dye provides the basis for quantitative interpretation of the data.

The 5' nuclease procedure is run on a real-time quantitative PCR device such as the ABI Prism 7700TM Sequence Detection. The system consists of a thermocycler, laser, charge-coupled device (CCD) camera and computer. The system amplifies samples in a 96-well format on a thermocycler. During amplification, laser-induced fluorescent signal is collected in real-time through fiber optics cables for all 96 wells, and detected at the CCD. The system includes software for running the instrument and for analyzing the data.

5' Nuclease assay data are initially expressed as Ct, or the threshold cycle. This is defined as the cycle at which the reporter signal accumulates above the background level of fluorescence. The ΔCt values are used as quantitative measurement of the relative number of starting copies of a particular target sequence in a nucleic acid sample when comparing cancer DNA results to normal human DNA results.

Table 4 describes the stage, T stage and N stage of various primary tumors which were used to screen the PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344, PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262 compounds of the invention.

Table 4
Primary Lung and Colon Tumor Profiles

Primary Tumor	Stage	Other Stage	Dukes Stage	T Stage	N Stage
Human lung tumor AdenoCa (SRCC724) [LT1]	IIA			T1	N1
Human lung tumor SqCCa (SRCC725) [LT1a]	IIB			T3	N0
Human lung tumor AdenoCa (SRCC726) [LT2]	IB			T2	N0
Human lung tumor AdenoCa (SRCC727) [LT3]	IIIA			T1	N2
Human lung tumor AdenoCa (SRCC728) [LT4]	IB			T2	N0
Human lung tumor SqCCa (SRCC729) [LT6]	IB			T2	N0
Human lung tumor Aden/SqCCa (SRCC730) [LT7]	IA			T1	N0
Human lung tumor AdenoCa (SRCC731) [LT9]	IB			T2	N0
Human lung tumor SqCCa (SRCC732) [LT10]	IIB			T2	N1
Human lung tumor SqCCa (SRCC733) [LT11]	IIA			T1	N1
Human lung tumor AdenoCa (SRCC734) [LT12]	IV			T2	N0
Human lung tumor AdenoSqCCa (SRCC735)[LT13]	IB			T2	N0
Human lung tumor SqCCa (SRCC736) [LT15]	IB			T2	N0
Human lung tumor SqCCa (SRCC737) [LT16]	IB			T2	N0
Human lung tumor SqCCa (SRCC738) [LT17]	IIB			T2	N1
Human lung tumor SqCCa (SRCC739) [LT18]	IB			T2	N0
Human lung tumor SqCCa (SRCC740) [LT19]	IB			T2	N0
Human lung tumor LCCa (SRCC741) [LT21]	IIB			T3	N1
Human lung AdenoCa (SRCC811) [LT22]	1A			T1	N0
Human colon AdenoCa (SRCC742) [CT2]		M1	D	pT4	N0
Human colon AdenoCa (SRCC743) [CT3]			B	pT3	N0
Human colon AdenoCa (SRCC 744) [CT8]			B	T3	N0
Human colon AdenoCa (SRCC745) [CT10]			A	pT2	N0
Human colon AdenoCa (SRCC746) [CT12]		MO, R1	B	T3	N0
Human colon AdenoCa (SRCC747) [CT14]		pMO, RO	B	pT3	pN0
Human colon AdenoCa (SRCC748) [CT15]		M1, R2	D	T4	N2
Human colon AdenoCa (SRCC749) [CT16]		pMO	B	pT3	pN0
Human colon AdenoCa (SRCC750) [CT17]			C1	pT3	pN1
Human colon AdenoCa (SRCC751) [CT1]		MO, R1	B	pT3	N0
Human colon AdenoCa (SRCC752) [CT4]			B	pT3	M0
Human colon AdenoCa (SRCC753) [CT5]		G2	C1	pT3	pN0
Human colon AdenoCa (SRCC754) [CT6]		pMO, RO	B	pT3	pN0
Human colon AdenoCa (SRCC755) [CT7]		G1	A	pT2	pN0
Human colon AdenoCa (SRCC756) [CT9]		G3	D	pT4	pN2
Human colon AdenoCa (SRCC757) [CT11]			B	T3	N0
Human colon AdenoCa (SRCC758) [CT18]		MO, RO	B	pT3	pN0

DNA Preparation:

DNA was prepared from cultured cell lines, primary tumors, and normal human blood. The isolation was performed using purification kit, buffer set and protease and all from Quiagen, according to the manufacturer's instructions and the description below.

Cell culture lysis:

Cells were washed and trypsinized at a concentration of 7.5×10^8 per tip and pelleted by centrifuging at 1000 rpm for 5 minutes at 4°C, followed by washing again with 1/2 volume of PBS and recentrifugation. The pellets were washed a third time, the suspended cells collected and washed 2x with PBS. The cells were then suspended into 10 ml PBS. Buffer C1 was equilibrated at 4°C. Qiagen protease #19155 was diluted into 6.25 ml

cold ddH_20 to a final concentration of 20 mg/ml and equilibrated at 4°C. 10 ml of G2 Buffer was prepared by diluting Qiagen RNAse A stock (100 mg/ml) to a final concentration of 200 μg/ml.

Buffer C1 (10 ml, 4°C) and ddH2O (40 ml, 4°C) were then added to the 10 ml of cell suspension, mixed by inverting and incubated on ice for 10 minutes. The cell nuclei were pelleted by centrifuging in a Beckman swinging bucket rotor at 2500 rpm at 4°C for 15 minutes. The supernatant was discarded and the nuclei were suspended with a vortex into 2 ml Buffer C1 (at 4°C) and 6 ml ddH_2O, followed by a second 4°C centrifugation at 2500 rpm for 15 minutes. The nuclei were then resuspended into the residual buffer using 200 μl per tip. G2 buffer (10 ml) was added to the suspended nuclei while gentle vortexing was applied. Upon completion of buffer addition, vigorous vortexing was applied for 30 seconds. Quiagen protease (200 μl, prepared as indicated above) was added and incubated at 50°C for 60 minutes. The incubation and centrifugation were repeated until the lysates were clear (*e.g.*, incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

Solid human tumor sample preparation and lysis:

Tumor samples were weighed and placed into 50 ml conical tubes and held on ice. Processing was limited to no more than 250 mg tissue per preparation (1 tip/preparation). The protease solution was freshly prepared by diluting into 6.25 ml cold ddH_2O to a final concentration of 20 mg/ml and stored at 4°C. G2 buffer (20 ml) was prepared by diluting DNAse A to a final concentration of 200 mg/ml (from 100 mg/ml stock). The tumor tissue was homogenated in 19 ml G2 buffer for 60 seconds using the large tip of the polytron in a laminar-flow TC hood in order to avoid inhalation of aerosols, and held at room temperature. Between samples, the polytron was cleaned by spinning at 2 x 30 seconds each in 2L ddH_20, followed by G2 buffer (50 ml). If tissue was still present on the generator tip, the apparatus was disassembled and cleaned.

Quiagen protease (prepared as indicated above, 1.0 ml) was added, followed by vortexing and incubation at 50°C for 3 hours. The incubation and centrifugation were repeated until the lysates were clear (*e.g.*, incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

Human blood preparation and lysis:

Blood was drawn from healthy volunteers using standard infectious agent protocols and citrated into 10 ml samples per tip. Quiagen protease was freshly prepared by dilution into 6.25 ml cold ddH_2O to a final concentration of 20 mg/ml and stored at 4°C. G2 buffer was prepared by diluting RNAse A to a final concentration of 200 μg/ml from 100 mg/ml stock. The blood (10 ml) was placed into a 50 ml conical tube and 10 ml C1 buffer and 30 ml ddH_2O (both previously equilibrated to 4°C) were added, and the components mixed by inverting and held on ice for 10 minutes. The nuclei were pelleted with a Beckman swinging bucket rotor at 2500 rpm, 4°C for 15 minutes and the supernatant discarded. With a vortex, the nuclei were suspended into 2 ml C1 buffer (4°C) and 6 ml ddH_2O (4°C). Vortexing was repeated until the pellet was white. The nuclei were then suspended into the residual buffer using a 200 μl tip. G2 buffer (10 ml) was added to the suspended nuclei while gently vortexing, followed by vigorous vortexing for 30 seconds. Quiagen protease was added (200 μl) and incubated at 50°C for 60 minutes. The incubation and centrifugation were repeated until the lysates were clear (*e.g.*, incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

Purification of cleared lysates:

(1) Isolation of genomic DNA:

Genomic DNA was equilibrated (1 sample per maxi tip preparation) with 10 ml QBT buffer. QF elution buffer was equilibrated at 50°C. The samples were vortexed for 30 seconds, then loaded onto equilibrated tips and drained by gravity. The tips were washed with 2 x 15 ml QC buffer. The DNA was eluted into 30 ml silanized, autoclaved 30 ml Corex tubes with 15 ml QF buffer (50°C). Isopropanol (10.5 ml) was added to each sample, the tubes covered with parafin and mixed by repeated inversion until the DNA precipitated. Samples were pelleted by centrifugation in the SS-34 rotor at 15,000 rpm for 10 minutes at 4°C. The pellet location was marked, the supernatant discarded, and 10 ml 70% ethanol (4°C) was added. Samples were pelleted again by centrifugation on the SS-34 rotor at 10,000 rpm for 10 minutes at 4°C. The pellet location was marked and the supernatant discarded. The tubes were then placed on their side in a drying rack and dried 10 minutes at 37°C, taking care not to overdry the samples.

After drying, the pellets were dissolved into 1.0 ml TE (pH 8.5) and placed at 50°C for 1-2 hours. Samples were held overnight at 4°C as dissolution continued. The DNA solution was then transferred to 1.5 ml tubes with a 26 gauge needle on a tuberculin syringe. The transfer was repeated 5x in order to shear the DNA. Samples were then placed at 50°C for 1-2 hours.

(2) Quantitation of genomic DNA and preparation for gene amplification assay:

The DNA levels in each tube were quantified by standard A_{260}/ A_{280} spectrophotometry on a 1:20 dilution (5 μl DNA + 95 μl ddH_2O) using the 0.1 ml quartz cuvettes in the Beckman DU640 spectrophotometer. A_{260}/A_{280} ratios were in the range of 1.8-1.9. Each DNA sample was then diluted further to approximately 200 ng/ml in TE (pH 8.5). If the original material was highly concentrated (about 700 ng/μl), the material was placed at 50°C for several hours until resuspended.

Fluorometric DNA quantitation was then performed on the diluted material (20-600 ng/ml) using the manufacturer's guidelines as modified below. This was accomplished by allowing a Hoeffer DyNA Quant 200 fluorometer to warm-up for about 15 minutes. The Hoechst dye working solution (#H33258, 10 μl, prepared within 12 hours of use) was diluted into 100 ml 1 x TNE buffer. A 2 ml cuvette was filled with the fluorometer solution, placed into the machine, and the machine was zeroed. pGEM 3Zf(+) (2 μl, lot #360851026) was added to 2 ml of fluorometer solution and calibrated at 200 units. An additional 2 μl of pGEM 3Zf(+) DNA was then tested and the reading confirmed at 400 +/- 10 units. Each sample was then read at least in triplicate. When 3 samples were found to be within 10% of each other, their average was taken and this value was used as the quantification value.

The fluorometricly determined concentration was then used to dilute each sample to 10 ng/μl in ddH_2O. This was done simultaneously on all template samples for a single TaqMan plate assay, and with enough material to run 500-1000 assays. The samples were tested in triplicate with Taqman™ primers and probe both B-actin and GAPDH on a single plate with normal human DNA and no-template controls. The diluted samples were used provided that the CT value of normal human DNA subtracted from test DNA was +/- 1 Ct. The diluted, lot-qualified genomic DNA was stored in 1.0 ml aliquots at -80°C. Aliquots which were subsequently to be used in the gene amplification assay were stored at 4°C. Each 1 ml aliquot is enough for 8-9 plates or 64 tests.

Gene amplification assay:

The PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344, PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262 compounds of the invention were screened in the following primary tumors and the resulting ΔCt values are reported in Tables 5A-5B.

Table 5A

ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell lines	PRO381	PRO1269	PRO1410	PRO1755	PRO1780	PRO1788	PRO3434	PRO1927	PRO3567	PRO1295
LT1	---	---	---	---	---	---	---	---	---	---
LT1-a	---	---	---	---	---	---	---	---	---	---
LT2	---	---	---	---	---	---	---	---	---	---
LT3	---	---	---	---	---	---	---	---	---	---
LT4	---	---	---	---	1.16	---	---	---	---	---
LT6	---	---	---	---	---	---	---	---	---	---
LT7	---	---	---	---	1.02	---	---	---	---	---
LT9	---	---	---	---	---	---	---	---	---	---
LT10	---	---	---	---	---	---	---	---	---	---
LT11	---	---	---	---	---	---	---	---	---	---
LT12	---	---	---	---	---	---	---	---	---	---
LT13	---	---	1.12 1.42	---	---	---	5.24 4.47	4.38 4.80	---	---
LT15	---	1.22	2.10 1.82	---	---	---	1.24	---	---	---

Table 5A Continued

<u>ΔCt values in lung and colon primary tumors and cell line models</u>

Primary Tumors or Cell lines	PRO381	PRO1269	PRO1410	PRO1755	PRO1780	PRO1788	PRO3434	PRO1927	PRO3567	PRO1295
LT16	---	1.14	1.44 1.45	1.36	---	---	3.65 3.19	2.73 2.74	---	---
LT17	---	1.26	---	---	---	---	---	---	---	---
LT18	---	---	---	1.18	---	---	---	---	---	---
LT19	---	---	---	---	---	---	---	---	---	---
LT21	---	---	---	---	---	---	---	---	---	---
CT2	---	---	2.36	2.35	---	---	---	---	---	---
CT3	---	---	1.09	---	---	1.35	---	---	---	---
CT8	---	---	---	1.64	---	1.26	---	---	---	---
CT10	---	---	1.41	2.05	---	1.37	---	---	---	---
CT12	---	---	---	1.15	---	1.24	---	---	---	---
CT14	---	---	1.46	1.40	---	2.58	---	---	---	---
CT15	---	---	---	---	---	---	1.19 1.40	1.10 1.30	---	---
CT16	---	---	---	---	---	---	---	---	---	---
CT17	---	---	---	---	---	---	---	---	---	---
CT1	---	---	---	---	---	1.09	---	---	---	---

Table 5A Continued

ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell lines	PRO381	PRO1269	PRO1410	PRO1755	PRO1780	PRO1788	PRO3434	PRO1927	PRO3567	PRO1295
CT4	---	---	---	---	---	1.22	---	---	---	---
CT5	---	---	2.14	---	---	---	---	---	---	---
CT6	---	---	---	---	---	---	---	---	---	---
CT7	---	---	---	---	---	---	---	---	---	---
CT9	---	---	---	---	---	1.52	---	---	---	---
CT11	---	---	1.29	---	---	---	---	---	---	---
CT18	---	---	---	---	---	---	---	---	---	---
HBL100	---	---	---	---	---	---	---	---	---	---
MB435s	---	---	---	---	---	---	---	---	---	---
T47D	---	---	---	---	---	---	---	---	---	---
MB468	---	---	---	---	---	-	---	---	---	---
MB175	---	---	---	---	---	---	---	---	---	---
MB361	---	---	---	---	---	---	---	---	---	---
BT20	---	---	---	---	---	---	---	---	---	---
MCF7	---	---	---	---	---	---	---	---	---	---
SKBR3	---	---	---	---	---	---	---	---	---	---

Table 5A Continued
ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell lines	PRO381	PRO1269	PRO1410	PRO1755	PRO1780	PRO1788	PRO3434	PRO1927	PRO3567	PRO1295
A549	---	---	---	---	---	---	1.09 1.51	---	---	---
Calu-1	---	---	---	---	---	---	---	---	---	---
Calu-6	---	---	---	---	---	---	1.60 1.22	---	---	---
H157	---	---	---	---	---	---	1.61	---	---	---
H441	---	---	---	---	---	---	1.07 1 15	---	---	---
H460	---	---	---	---	---	---	1.01	---	---	---
SKMES1	---	---	---	---	---	---	1.02	---	---	---
SW900	---	---	---	---	---	---	---	---	---	---
SW480	---	---	---	---	---	---	---	---	---	---
SW620	---	---	---	---	---	---	1 30	---	---	---
Colo320	---	---	---	---	---	---	1.78 1.76 1.74	1.51	2.31	---
HT29	---	---	1.22	---	---	---	1.64	---	---	---
HM7	---	---	---	---	---	---	---	---	---	---
WiDr	---	---	---	---	---	---	---	---	---	---

Table 5A Continued
ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell lines	PRO381	PRO1269	PRO1410	PRO1755	PRO1780	PRO1788	PRO3434	PRO1927	PRO3567	PRO1295
HCT116	---	---	---	---	---	---	2.15 2.22	1.41 1.47	---	---
SKC01	---	---	---	---	---	---	---	---	---	---
SW403	---	---	---	---	---	---	1.75	---	1.07	---
LS174T	---	---	---	---	---	---	1.42	---	1.17	---
LT22	---	---	---	1.26	1.07	---	---	---	---	---
LT8	---	---	---	---	---	---	---	---	---	---
Colo205	---	---	---	---	---	---	---	---	---	---
HCT15	---	---	---	---	---	---	---	---	---	---
HCC2998	---	---	---	---	---	---	1.15	---	---	---
KM12	---	---	---	---	---	---	---	---	---	---
H522	---	---	---	---	---	---	---	---	---	---
H810	---	---	---	---	---	---	1.20 1.54	---	---	---
LT26	---	---	---	---	---	---	---	---	---	---
LT27	---	---	---	---	---	---	---	---	---	---
LT28	---	---	---	---	---	---	---	---	---	---

Table 5A Continued
ACt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell lines	PRO381	PRO1269	PRO1410	PRO1755	PRO1780	PRO1788	PRO3434	PRO1927	PRO3567	PRO1295
LT29	---	---	---	---	---	---	---	---	---	---
LT30	---	---	---	---	---	---	---	---	---	---
LT31	---	---	---	---	---	---	---	---	---	---
LT33	---	---	---	---	---	---	---	---	---	---
CT25	---	---	---	---	---	---	---	---	---	---
CT28	---	---	---	---	---	---	---	---	---	---
CT35	---	---	---	---	---	---	---	---	---	---
HF-000716	---	---	---	---	---	---	---	---	---	---
HF-000733	---	---	---	---	---	---	---	---	---	---
HF-000831	---	---	---	---	---	---	---	---	---	---
HF-000832	---	---	---	---	---	---	---	---	---	---
HF-000613	---	---	---	---	---	---	---	---	---	---
HF-000499	---	---	---	---	---	---	---	---	---	---
HF-000539	---	---	---	---	---	---	---	---	---	1.49
HF-000575	---	---	---	---	---	---	---	---	---	---
HF-000698	---	---	---	---	---	---	---	---	---	1.09

Table 5A Continued
ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell lines	PRO381	PRO1269	PRO1410	PRO1755	PRO1780	PRO1788	PRO3434	PRO1927	PRO3567	PRO1295
HF-000545	---	---	---	---	---	---	---	---	---	1.11
SRCC1094	---	---	---	---	---	---	---	---	---	---
SRCC1095	---	---	---	---	---	---	---	---	---	---
SRCC1096	---	---	---	---	---	---	---	---	---	---
SRCC1097	---	---	---	---	---	---	---	---	---	---
SRCC1098	---	---	---	---	---	---	---	---	---	---
SRCC1099	---	---	---	---	---	---	---	---	---	---
SRCC1100	---	---	---	---	---	---	---	---	---	---
SRCC1101	---	---	---	---	---	---	---	---	---	---
HF-000631	---	---	---	---	---	---	---	---	---	1.27
HF-000641	---	---	---	---	---	---	---	---	---	---
HF-000643	4.83	---	---	---	---	---	---	---	---	---
HF-000840	1.08	---	---	---	---	---	---	---	---	1.97
HF-000842	---	---	---	---	---	---	2.20	2.41	---	---
HF-000762	---	---	---	---	---	---	---	---	---	---
HF-000789	---	---	---	---	---	---	---	---	---	---

Table 5A Continued
ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell lines	PRO381	PRO1269	PRO1410	PRO1755	PRO1780	PRO1788	PRO3434	PRO1927	PRO3567	PRO1295
HF-000795	---	---	---	---	---	---	---	---	---	---
HF-000811	2.09 3.15	---	---	---	---	---	---	---	---	---
HF-001294	1.14 1.08	---	---	---	---	---	1.17	2.31	---	---
HF-001293	---	---	---	---	---	---	---	---	---	---
HF-001300	---	---	---	---	---	---	---	---	---	---
HF-001297	---	---	---	---	---	---	---	---	---	---
HF-001295	---	---	---	---	---	---	---	---	---	---
HF-001299	---	---	---	---	---	---	1.11	2.40	---	---
HF-001296	3.18 3.53	---	---	---	---	---	4.64	5.14	---	---
HF-001291	1.17	---	---	---	---	---	---	---	---	---
HF-000842	---	---	---	---	---	---	---	---	---	---

Table 5B

<u>ΔCt values in lung and colon primary tumors and cell line models</u>

Primary Tumors or Cell Lines	PRO1293	PRO1303	PRO4344	PRO4354	PRO4397	PRO4407	PRO1555	PRO1096	PRO2038	PRO2262
LT1	---	---	---	---	---	---	---	---	---	---
LT1-a	---	---	---	---	---	---	---	---	1.25	---
LT2	---	---	---	---	---	---	---	---	---	---
LT3	---	---	---	---	---	---	---	---	---	---
LT4	---	---	---	---	---	---	---	---	---	---
LT6	---	---	---	---	---	---	---	---	1.20	---
LT7	---	---	---	---	---	---	---	---	1.66	---
LT9	---	---	---	---	---	---	---	---	---	---
LT10	---	---	---	---	---	---	---	---	---	---
LT11	---	---	---	---	---	---	---	---	---	---
LT12	---	---	---	---	---	---	---	---	1.27	---
LT13	---	1.42	---	---	---	---	4 20 4.45	---	---	---
LT15	---	1.17	---	---	---	---	1.36 1.15	---	---	---
LT16	---	1.42	---	---	---	---	3.71 3.99	---	---	---
LT17	---	---	---	---	---	---	---	---	---	---

Table 5B
ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell Lines	PRO1293	PRO1303	PRO4344	PRO4354	PRO4397	PRO4407	PRO1555	PRO1096	PRO2038	PRO2262
LT18	---	---	---	---	---	---	---	---	---	---
LT19	---	---	---	---	---	---	---	---	---	---
LT21	---	---	---	---	---	---	---	---	---	---
CT2	---	---	---	---	---	---	---	---	---	---
CT3	---	---	---	---	---	---	---	---	---	---
CT8	---	---	---	---	---	---	---	---	---	---
CT10	---	---	---	---	---	---	---	---	---	---
CT12	---	---	---	---	---	---	---	---	---	---
CT14	---	---	---	---	---	---	---	---	---	---
CT15	---	---	---	---	---	---	1.34 1.62	---	---	---
CT16	---	1.13	---	---	---	---	1.04 1.05	---	---	---
CT17	---	---	---	---	---	---	1.16	---	---	---
CT1	---	---	---	---	---	---	---	---	---	---
CT4	---	---	---	---	---	---	---	---	---	---
CT5	---	---	---	---	---	---	---	---	---	---
CT6	---	---	---	---	---	---	---	---	---	---

Table 5B Continued

ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell Lines	PRO1293	PRO1303	PRO4344	PRO4354	PRO4397	PRO4407	PRO1555	PRO1096	PRO2038	PRO2262
CT7	---	---	---	---	---	---	---	---	---	---
CT9	---	---	---	---	---	---	---	---	---	---
CT11	---	---	---	---	---	---	---	---	---	---
CT18	---	---	---	---	---	---	---	---	---	---
HBL100	---	---	---	---	---	---	---	1.10	---	---
MB435s	---	---	---	---	---	---	---	---	---	---
T47D	---	---	---	---	---	---	---	1.37	---	---
MB468	---	---	---	---	---	---	---	---	---	---
MB175	---	---	---	---	---	---	---	---	---	---
MB361	---	---	---	---	---	---	---	---	---	---
BT20	---	---	---	---	---	---	---	---	---	---
MCF7	---	---	---	---	---	---	---	---	---	---
SKBR3	---	---	---	---	---	---	---	---	---	---
A549	---	1.20	---	---	---	---	2.17 2.11	---	---	---
Calu-1	---	---	---	---	---	---	1.39	---	---	---
Calu-6	---	---	---	---	---	---	1.12	---	---	---
H157	---	---	---	---	---	---	---	---	---	---

Table 5B Continued
ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell Lines	PRO1293	PRO1303	PRO4344	PRO4354	PRO4397	PRO4407	PRO1555	PRO1096	PRO2038	PRO2262
H441	---	---	---	---	---	---	2.06	---	---	---
H460	---	---	---	---	---	---	1.88	---	---	---
SKMES1	---	---	---	---	---	---	1.90	---	---	---
SW900	---	---	---	---	---	---	---	---	---	---
SW480	---	---	---	---	---	---	---	1.41	---	---
SW620	---	---	---	---	---	---	2.24	---	---	---
Colo320	---	---	---	---	---	---	2.21 2.24	1.72	---	---
HT29	---	---	---	---	---	---	---	1.32	---	---
HM7	---	---	---	---	---	---	---	---	---	---
WiDr	---	---	---	---	---	---	---	1.57	---	---
HCT116	---	---	---	---	---	---	2.46 2.66	1.06	---	---
SKC01	---	---	---	---	---	---	---	1.01	---	---
SW403	---	---	---	---	---	---	---	1.43	---	---
LS174T	---	---	---	---	---	---	---	---	---	---
LT22	---	---	---	---	---	---	---	---	---	---

Table 5B Continued

ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell Lines	PRO1293	PRO1303	PRO4344	PRO4354	PRO4397	PRO4407	PRO1555	PRO1096	PRO2038	PRO2262
LT8	---	---	---	---	---	---	---	---	1.74	---
Colo205	---	---	---	---	---	---	---	---	---	---
HCT15	---	---	---	---	---	---	---	---	---	---
HCC2998	---	---	---	---	---	---	---	---	---	---
KM12	---	---	---	---	---	---	---	---	---	---
H522	---	---	---	---	---	---	---	---	---	---
H810	---	---	---	---	---	---	---	---	---	---
LT25	---	---	---	---	---	---	---	---	---	---
LT26	---	---	---	---	---	---	---	---	1.01	---
LT27	---	---	---	---	---	---	---	---	---	---
LT28	---	---	---	---	---	---	---	---	1.32	---
LT29	---	---	---	---	---	---	---	---	---	---
LT30	---	---	---	---	---	---	---	---	---	---
LT31	---	---	---	---	---	---	---	---	---	---
LT32	---	---	---	---	---	---	---	---	---	---
LT33	---	---	---	---	---	---	---	---	---	---
CT19	---	---	---	---	---	---	---	---	---	---

Table 5B Continued
ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell Lines	PRO1293	PRO1303	PRO4344	PRO4354	PRO4397	PRO4407	PRO1555	PRO1096	PRO2038	PRO2262
CT20	---	---	---	---	---	---	---	---	---	---
CT21	---	---	---	---	---	---	---	---	---	---
CT22	---	---	---	---	---	---	---	---	---	---
CT23	---	---	---	---	---	---	---	---	---	---
CT24	---	---	---	---	---	---	---	---	---	---
CT25	---	---	---	---	---	---	---	---	---	---
CT26	---	---	---	---	---	---	---	---	---	---
CT27	---	---	---	---	---	---	---	---	---	---
CT28	---	---	---	---	---	---	---	---	---	---
CT29	---	---	---	---	---	---	---	---	---	---
CT30	---	---	---	---	---	---	---	---	---	---
CT31	---	---	---	---	---	---	---	---	---	---
CT32	---	---	---	---	---	---	---	---	---	---
CT33	---	---	---	---	---	---	---	---	---	---
CT35	---	---	---	---	---	---	---	---	---	---
CT36	---	---	---	---	---	---	---	---	---	---
HF-000716	---	---	---	---	---	---	2.63 2.73	---	---	1.68

Table 5B Continued

ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell Lines	PRO1293	PRO1303	PRO4344	PRO4354	PRO4397	PRO4407	PRO1555	PRO1096	PRO2038	PRO2262
HF-000733	---	---	---	---	---	---	2.58 2.71 1.39	---	---	2.04
HF-000831	---	---	---	---	---	---	---	---	---	---
HF-000832	---	---	---	1.10	---	1.31 1.18	---	---	---	1.13
HF-000611	---	---	---	---	---	---	4.99	---	---	---
HF-000613	---	---	---	---	---	---	---	---	---	---
HF-000499	---	---	---	---	---	---	---	---	---	---
HF-000539	2.33	---	1.32	1.39 1.09	---	1.79 1.61	3.13 2.55	---	---	2.03 1.86
HF-000575	---	---	1.21	1.14	---	1.48 1.25	1.32	---	---	1.16
HF-000698	---	---	---	1.14	---	1.14	---	---	---	1 40 1.22
HF-000545	---	---	1.62	2.12 1.72	---	1 52 1.63	1.59 1.68	---	---	1.17
SRCC1094	---	---	---	---	---	---	---	---	---	---
SRCC1095	---	---	---	---	---	---	---	---	---	---
SRCC1096	---	---	---	---	---	---	---	---	---	---
SRCC1097	---	---	---	---	---	---	---	---	---	---

Table 5B Continued

ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell Lines	PRO1293	PRO1303	PRO4344	PRO4354	PRO4397	PRO4407	PRO1555	PRO1096	PRO2038	PRO2262
SRCC1098	---	---	---	---	---	---	---	---	1.24	---
SRCC1099	---	---	---	---	---	---	---	---	---	---
SRCC1100	---	---	---	---	---	---	---	---	---	---
SRCC1101	---	---	---	---	---	---	---	---	---	---
HF-000631	---	---	---	---	--	---	1.37	---	---	---
HF-000641	---	---	---	---	---	---	---	---	---	---
HF-000643	---	---	---	---	---	---	---	---	---	---
HF-000840	1.71	---	1.03	---	2.09	1.50 1 42	3.63	---	---	1.49 1.49
HF-000842	---	---	---	---	---	1.24	1.99	--	---	---
HF-000762	---	---	---	1.39 1.15	---	---	---	---	---	---
HF-000789	---	---	---	---	---	---	---	---	---	---
HF-000795	1.13	---	1.12	---	---	1.02 1.04	---	---	---	---
HF-000811	---	---	---	---	---	---	---	---	---	---
HF-001291	---	---	---	---	---	---	---	---	---	---
HF-001293	---	---	---	---	---	---	---	---	---	---

Table 5B Continued
ΔCt values in lung and colon primary tumors and cell line models

Primary Tumors or Cell Lines	PRO1293	PRO1303	PRO4344	PRO4354	PRO4397	PRO4407	PRO1555	PRO1096	PRO2038	PRO2262
HF-001294	---	---	---	---	---	---	---	---	---	---
HF-001295	---	---	---	---	---	---	---	---	---	---
HF-001296	---	---	---	2.11	---	2.21	---	---	---	2.18
HF-001297	---	---	---	---	---	---	---	---	---	---
HF-001299	---	---	---	---	---	---	---	---	---	---
HF-001300	---	---	---	---	---	---	---	---	---	---

PRO3434:

PRO3434 (DNA77631-2537) was reexamined along with selected tumors from the above initial screen with epicenter mapping. Table 6 describes the epicenter markers that were employed in association with PRO3434 (DNA77631-2537). These markers are located in close proximity to DNA77631 and are used to assess the amplification status of the region of Chromosome 7 in which DNA77631 is located. The distance between markers is measured in centirays (cR), which is a radiation breakage unit approximately equal to a 1% chance of a breakage between two markers. One cR is very roughly equivalent to 20 kilobases. The marker sWSS918 is the marker found to be the closest to the location on Chromosome 7 where DNA77631-2537 closely maps.

Table 7 indicates the ΔCt values for results of epicenter mapping relative to DNA77631, indicating the relative amplification in the region more immediate to the actual location of DNA77631 along Chromosome 7.

Table 6

Epicenter Markers Along Chromosome 7 Used for DNA77631

Map Position on Chromosome 7	Stanford Human Genome Center Marker Name	Distance to Next Marker (cR)
G1	SHGC-34913	17
G2	AFMa090xg1	25
G3	SHGC-10715	16
G4	SHGC-34866	5
G5	SHGC-32510	48
DNA 77631	-	-
G6	sWSS918	19
G7	AFMc027xb5	-

Table 7

Amplification of Epicenter Markers Relative to DNA77631 (ΔCt)

Tumor	G1	G2	G3	G4	G5	DNA 77631	G6	G7
HF-000613	1.31	0.12	0.61	0.46	0.38	-0.28	0.48	0.58
HF-000545	1.55	0.55	0.09	0.16	0.27	0.69	0.26	0.25
HF-000539	1.53	-1.68	0.88	0.76	0.79	2.75	0.98	0.96
HF-000575	1.47	1.02	0.02	-0.17	0.15	0.48	0.20	0.33
HF-000698	0.73	-0.31	-0.08	-0.27	-0.07	-0.26	-0.21	-0.10
HF-000499	0.67	-0.05	0.04	0.12	0.23	-0.30	-0.14	0.21
HF-000733	1.08	1.19	0.41	0.39	0.46	3.00	0.51	0.68
HF-000716	0.65	0.56	-0.41	-0.02	-0.13	2.59	-0.23	0.01

DISCUSSION AND CONCLUSION:

PRO381 (DNA44194-1317):

The ΔCt values for DNA44194-1317 in a variety of tumors are reported in Table 5A. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5A indicates that significant amplification of nucleic acid DNA44194-1317 encoding PRO381 occurred: (1) in primary lung tumors: HF-000643, HF-000840, HF-001291, HF-001294, and HF-001296; and (2) in colon tumor center HF-000811. Because amplification of DNA44194-1317 occurs in various tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA44194-1317 (PRO381) would be expected to have utility in cancer therapy.

PRO1269 (DNA66520-1536):

The ΔCt values for DNA66520-1536 in a variety of tumors are reported in Table 5A. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5A indicates that significant amplification of nucleic acid DNA66520-1536 encoding PRO1269 occurred in primary lung tumors: LT15, LT16 and LT17. Because amplification of DNA66520-1536 occurs in various lung tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA66520-1536 (PRO1269) would be expected to have utility in cancer therapy.

PRO1410 (DNA68874-1622):

The ΔCt values for DNA68874-1622 in a variety of tumors are reported in Table 5A. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5A indicates that significant amplification of nucleic acid DNA68874-1622 encoding PRO1410 occurred: (1) in primary lung tumors: LT13, LT15 and LT16; (2) in primary colon tumors: CT2, CT3, CT5, CT10, CT11, and CT14; and (3) in colon cell line HT29. Because amplification of DNA68874-1622 occurs in various lung and colon tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA68874-1622 (PRO1410) would be expected to have utility in cancer therapy.

PRO1755 (DNA76396-1698):

The ΔCt values for DNA76396-1698 in a variety of tumors are reported in Table 5A. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5A indicates that significant amplification of nucleic acid DNA76396-1698 encoding PRO1755 occurred: (1) in primary lung tumors: LT16, LT18 and LT22; and (2) in primary colon tumors: CT2, CT8, CT10, CT12, and CT14. Because amplification of DNA76396-1698 occurs in various lung and colon tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA76396-1698 (PRO1755) would be expected to have utility in cancer therapy.

PRO1780 (DNA71169-1709):

The ΔCt values for DNA71169-1709 in a variety of tumors are reported in Table 5A. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5A indicates that significant amplification of nucleic acid DNA71169-1709 encoding PRO1780 occurred in primary lung tumors: LT4, LT7 and LT22. Because amplification of DNA71169-1709 occurs in various lung tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA71169-1709 (PRO1780) would be expected to have utility in cancer therapy.

PRO1788 (DNA77652-2505):

The ΔCt values for DNA77652-2505 in a variety of tumors are reported in Table 5A. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5A indicates that significant amplification of nucleic acid DNA77652-2505 encoding PRO1788 occurred in primary colon tumors: CT1, CT3, CT4, CT8, CT9, CT10, CT12, and CT14. Because amplification of DNA77652-2505 occurs in various colon tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA77652-2505 (PRO1788) would be expected to have utility in cancer therapy.

PRO3434 (DNA77631-2537):

The ΔCt values for DNA77631-2537 in a variety of tumors are reported in Table 5A. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5A indicates that significant amplification of nucleic acid DNA77631-2537 encoding PRO3434 occurred: (1) in primary lung tumors: LT13, LT15, LT16, HF-000842, HF-001294, HF-001296, and HF-001299; (2) in lung cell lines: A549, Calu-6, H157, H441, H460, SKMES1, and H810; (3) in primary colon tumor CT15; and (4) in colon cell lines: SW620, Colo320, HT29, HCT116, SW403, LS174T, and HCC2998.

Amplification has been confirmed by epicenter mapping (Table 7) for DNA77631-2537 and resulted in significant amplification: (1) in primary colon tumor HF-000539; and (2) in testis tumor center HF-000733 and testis tumor margin HF-000716. In contrast, the amplification of the closest known epicenter markers does not occur to a greater extent than that of DNA77631 (Table 7). This strongly suggests that DNA77631-1317 is the gene responsible for the amplification of the particular region on Chromosome 7.

Because amplification of DNA77631 occurs in various tumors including colon and testis tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA77631-2537 (PRO3434) would be expected to have utility in cancer therapy.

PRO1927 (DNA82307-2531):

The ΔCt values for DNA82307-2531 in a variety of tumors are reported in Table 5A. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5A indicates that significant amplification of nucleic acid DNA82307-2531 encoding PRO1927 occurred: (1) in primary lung tumors: LT13, LT16, HF-000842, HF-001294, HF-001296, and HF-001299; (2) in primary colon tumor CT15; and (3) in colon cell lines: Colo320 and HCT116. Because amplification of DNA occurs in various lung and colon tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA82307-2531 (PRO1927) would be expected to have utility in cancer therapy.

PRO3567 (DNA56049-2543):

The ΔCt values for DNA56049-2543 in a variety of tumors are reported in Table 5A. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5A indicates that significant amplification of nucleic acid DNA56049-2543 encoding PRO3567 occurred in colon cell lines: Colo320, SW403 and LS174T. Because amplification of DNA56049-2543 occurs in various colon cell lines, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA56049-2543 (PRO3567) would be expected to have utility in cancer therapy.

PRO1295 (DNA59218-1559):

The ΔCt values for DNA59218-1559 in a variety of tumors are reported in Table 5A. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5A indicates that significant amplification of nucleic acid DNA59218-1559 encoding PRO1295 occurred: (1) in primary lung tumors: HF-000631 and HF-000840; (2) colon tumor centers: HF-000539 and HF-000698; and (3) in breast tumor center HF-000545. Because amplification of DNA59218-1559 occurs in various tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA59218-1559 (PRO1295) would be expected to have utility in cancer therapy.

PRO1293 (DNA60618-1557):

The ΔCt values for DNA60618-1557 in a variety of tumors are reported in Table 5B. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5B indicates that significant amplification of nucleic acid DNA60618-1557 encoding PRO1293 occurred: (1) in primary lung tumor HF-000840; and (2) in colon tumor centers: HF-000539 and HF-000795. Because amplification of DNA60618-1557 occurs in various lung and colon tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA60618-1557 (PRO1293) would be expected to have utility in cancer therapy.

PRO1303 (DNA65409-1566):

The ΔCt values for DNA65409-1566 in a variety of tumors are reported in Table 5B. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5B indicates that significant amplification of nucleic acid DNA65409-1566 encoding PRO1303 occurred: (1) in primary lung tumors: LT13, LT15 and LT16; (2) in lung cell line A549; and (3) in colon tumor CT16. Because amplification of DNA65409-1566 occurs in various tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA65409-1566 (PRO1566) would be expected to have utility in cancer therapy.

PRO4344 (DNA84927-2585):

The ΔCt values for DNA84927-2585 in a variety of tumors are reported in Table 5B. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5B indicates that significant amplification of nucleic acid DNA84927-2585 encoding PRO4344 occurred: (1) in primary lung tumor HF-000840; (2) in colon tumor centers: HF-000539, HF-000575 and HF-000795; and (3) in breast tumor center HF-000545. Because amplification of DNA84927-2585 occurs in various tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA84927-2585 (PRO4344) would be expected to have utility in cancer therapy.

PRO4354 (DNA92256-2596):

The ΔCt values for DNA92256-2596 in a variety of tumors are reported in Table 5B. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5B indicates that significant amplification of nucleic acid DNA92256-2596 encoding PRO4354 occurred: (1) in primary lung tumor HF-001296; (2) in colon tumor centers: HF-000539, HF-000575, HF-000698, and HF-000762; (3) in breast tumor center HF-000545; and (4) in parathyroid tumor HF-000832. Because amplification of DNA92256-2596 occurs in various tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA92256-2596 (PRO4354) would be expected to have utility in cancer therapy.

PRO4397 (DNA83505-2606):

The ΔCt values for DNA83505-2606 in a variety of tumors are reported in Table 5B. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5B indicates that significant amplification of nucleic acid DNA83505-2606 encoding PRO4397occurred in primary lung tumor HF-000840. Because amplification of DNA83505-2606 occurs in lung tumor, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA83505-2606 (PRO4397) would be expected to have utility in cancer therapy.

PRO4407 (DNA92264-2616):

The ΔCt values for DNA92264-2616 in a variety of tumors are reported in Table 5B. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5B indicates that significant amplification of nucleic acid DNA92264-2616 encoding PRO4407 occurred: (1) in primary lung tumors HF-000840, HF-000842 and HF-001296; (2) in colon tumor centers: HF-000539, HF-000575, HF-000698, and HF-000795; (3) in breast tumor HF-000545; and (4) in parathyroid tumor HF-000832. Because amplification of DNA92264-2616 occurs in various tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA92264-2616 (PRO2616) would be expected to have utility in cancer therapy.

PRO1555 (DNA73744-1665):

The ΔCt values for DNA73744-1665 in a variety of tumors are reported in Table 5B. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5B indicates that significant amplification of nucleic acid DNA73744-1665 encoding PRO1555 occurred: (1) in primary lung tumors: LT13, LT15, LT16, HF-000631, HF-000840, and HF-000842; (2) in lung cell lines: A549, Calu-1, Calu-6, H441, H460, and SKMES1; (3) in primary colon tumors: CT15, CT16, CT17, and colon tumor centers HF-000539 and HF-000575; (4) in colon cell lines: SW620, Colo320 and HCT116; (5) in breast tumor center HF-000545; (6) in kidney tumor center HF-000611; and (7) in testis tumor margin HF-000716 and testis tumor center HF-000733. Because amplification of DNA73744-1665 occurs in various tumors, it is highly

probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA73744-1665 (PRO1555) would be expected to have utility in cancer therapy.

PRO1096 (DNA61870):

The ΔCt values for DNA61870 in a variety of tumors are reported in Table 5B. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5B indicates that significant amplification of nucleic acid DNA61870 encoding PRO1096 occurred: (1) in colon cell lines: SW480, Colo320, HT29, WiDr, HCT116, SKCO1, and SW403; and (2) in breast cell lines HBL100 and T47D. Because amplification of DNA61870 occurs in various tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA61870 (PRO1096) would be expected to have utility in cancer therapy.

PRO2038 (DNA83014):

The ΔCt values for DNA83014 in a variety of tumors are reported in Table 5B. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5B indicates that significant amplification of nucleic acid DNA83014 encoding PRO2038 occurred: (1) in primary lung tumors: LT1a, LT6, LT7, LT8, LT12, LT26, and LT28; and (2) in breast tumor SRCC1098. Because amplification of DNA83014 occurs in various tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA83014 (PRO2038) would be expected to have utility in cancer therapy.

PRO2262 (DNA88273):

The ΔCt values for DNA88273 in a variety of tumors are reported in Table 5B. A ΔCt of >1 was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 5B indicates that significant amplification of nucleic acid DNA88273 encoding PRO2262 occurred: (1) in primary lung tumors: HF-000840 and HF-001296; (2) in primary colon tumors: HF-000539, HF-000575 and HF-000698; (3) in breast tumor center HF-000545; (4) in testis tumor margin HF-000716 and testis tumor center HF-000733; and (5) in parathyroid tumor HF-000832. Because amplification of DNA88273 occurs in various tumors, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA88273 (PRO2262) would be expected to have utility in cancer therapy.

EXAMPLE 21

Use of PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344, PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262 as a hybridization probe

The following method describes use of a nucleotide sequence encoding a PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344,

PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262 polypeptide as a hybridization probe.

DNA comprising the coding sequence of a full-length or mature "PRO" polypeptide as disclosed herein and/or fragments thereof may be employed as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants of PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344, PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262) in human tissue cDNA libraries or human tissue genomic libraries.

Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of radiolabeled PRO381-, PRO1269-, PRO1410-, PRO1755-, PRO1780-, PRO1788-, PRO3434-, PRO1927-, PRO3567-, PRO1295-, PRO1293-, PRO1303-, PRO4344-, PRO4354-, PRO4397-, PRO4407-, PRO1555-, PRO1096-, PRO2038- or PRO2262-derived probe to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344, PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262 can then be identified using standard techniques known in the art.

EXAMPLE 22

Expression of PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344, PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262 Polypeptides in *E. coli*.

This example illustrates preparation of an unglycosylated form of PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344, PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262 by recombinant expression in *E. coli*.

The DNA sequence encoding the PRO polypeptide of interest is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; *see* Bolivar *et al.*, Gene, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a trp promoter, a poly-His leader (including the first six STII codons, poly-His sequence, and enterokinase cleavage site), the PRO381, PRO1269, PRO1410, PRO1755, PRO1780, PRO1788, PRO3434, PRO1927, PRO3567, PRO1295, PRO1293, PRO1303, PRO4344, PRO4354, PRO4397, PRO4407, PRO1555, PRO1096, PRO2038 or PRO2262 coding region, lambda transcriptional terminator, and an argU gene.